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PREPRINT

THE DRY AEROSOL DEPOSITION DEVICE (DADD): AN INSTRUMENT FOR DEPOSITING MICROBIAL AEROSOLS ONTO SURFACES

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14. ABSTRACT

Contamination of infrastructure and equipment with biowarfare agents has led to the development of antimicrobial surfaces/coatings that are designed to "self-sterilize". Surfaces will likely be contaminated via an aerosol exposure and thus antimicrobial efficacy measurements should also be performed using biological aerosols. Standard tests that use microbial agents suspended in aqueous buffers may provide misleading results that overestimate the performance of the surface. A settling chamber is the most common instrument for applying biological aerosols to surfaces. However, settling chambers have some drawbacks that make them undesirable for all applications (i.e., slow loading times, large footprint, variable loading, etc.). We developed a Dry Aerosol Deposition Device (DADD) that uses impaction rather than settling for loading surfaces with biological aerosols. The use of impaction allows for rapid and highly reproducible loading of microorganisms onto surfaces. We demonstrated that the DADD can deliver both *Bacillus atrophaeus* spores and *Staphylococcus aureus* vegetative cells to glass coupons at concentrations exceeding $1X10^4$ CFU/cm^2. The coefficient of variation (CV) for sample-to-sample loading within an experiment was 13.6% for spores and 6.1% for *S. aureus* cells. The DADD is also a relatively simple and inexpensive device that can easily be contained within a 4' biological safety cabinet.

15. SUBJECT TERMS

bioaerosol, deposition, method, microorganism, particle, spore

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Title

The Dry Aerosol Deposition Device (DADD): An Instrument for Depositing Microbial Aerosols onto Surfaces

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Abstract

Contamination of infrastructure and equipment with biowarfare agents has led to the development of antimicrobial surfaces/coatings that are designed to “self-sterilize.” Surfaces will likely be contaminated via an aerosol exposure and thus antimicrobial efficacy measurements should also be performed using biological aerosols. Standard tests that use microbial agents suspended in aqueous buffers may provide misleading results that overestimate the performance of the surface. A settling chamber is a common instrument for applying biological aerosols to surfaces. However, settling chambers have some drawbacks that make them undesirable for all applications (i.e., slow loading times, large footprint, variable loading, etc.). We developed a Dry Aerosol Deposition Device (DADD) that uses impaction rather than settling for loading surfaces with biological aerosols. The use of impaction allows for rapid and highly reproducible loading of microorganisms onto surfaces. We demonstrated that the DADD can deliver both *Bacillus atrophaeus* spores and *Staphylococcus aureus* vegetative cells to glass coupons at concentrations exceeding $1X10^4$ CFU/cm^2. The coefficient of variation (CV) for sample-to-sample loading within an experiment was 13.6% for spores and 6.1% for *S. aureus* cells. The DADD is also a relatively simple and inexpensive device that can easily be contained within a 4-foot biological safety cabinet.

Key words

bioaerosol, deposition, method, microorganism, particle, spore

Introduction

The methods used to measure antimicrobial efficacy of decontamination technologies are, in principle, very simple: samples are contaminated with microorganisms, a subset is exposed to a decontaminant and a control group is not exposed to the decontaminant. Microbes are extracted and then quantified using viable plating; the antimicrobial efficacy is the ratio of the two values. Many standard tests (American Association of Textile Colorists and Chemists (AATCC) 144, American Society for Testing and Materials (ASTM) E2148, E2414) use some variation of this strategy to provide the efficacy of the decontamination under a given set of conditions. However, the conditions used to measure antimicrobial efficacy may influence the susceptibility of the microorganisms to the disinfection technologies (Betty et al., 2005, Lai et al., 2004, Prugh and Calomiris, 2006). For example, organic components are known to compete with oxidative agents (Betty et al., 2005) and reduce the effectiveness of ultraviolet light (Lai et al., 2004). Properties such as temperature, relative humidity, presence of organic material, presence of a carrier, water content, etc., must be considered to ensure the laboratory test simulates field-based exposures. Only then can laboratory data be extrapolated to field-based efficacy.

A major concern during the last decade is the deployment of biological warfare agents. Although the primary effect of biological agents is causing infections, a secondary consequence is contamination of infrastructure. This was clearly demonstrated during the 2001 anthrax attacks, in which multiple buildings were closed for months to allow for decontamination (Canter, 2004). Biological warfare agents are expected to be delivered

in aerosol form; therefore, a biological aerosol is an appropriate challenge to evaluate decontamination technologies. Biological aerosols are complex entities and changes in their particle size or the presence of inert materials may influence their susceptibility to the decontaminant. Another important factor affecting biological aerosols is their low water content. Water quickly evaporates from aerosol droplets, leaving droplet nuclei. The droplet nuclei are responsible for contaminating surfaces; thus droplet nuclei must be used to evaluate decontamination technologies.

No standard test methods exist for applying biological aerosols to surfaces. The likely reason is that the application of biological aerosols to surfaces is very challenging. In liquid systems, it is quite simple to apply a standard inoculum of microorganisms to surfaces. However, quantifiably adding biological aerosols to surfaces requires special instrumentation and expertise in aerosols. The commonest device for applying aerosols to surfaces is a settling chamber (Barron et al., 2008, Brown et al., 2007, Feather and Chen, 2003, Marple and Rubow, 1983, McCready, 1986). A settling chamber provides a realistic challenge and, if controlled properly, can produce reproducible results. However, one drawback to using a settling chamber is the time required to load samples. Most protocols call for loading times ranging from 10–24 hours and, depending on the requirements for the challenge, this time may be unacceptable. Also larger particles settle more quickly than smaller particles, which may cause problems when evaluating self-decontaminating surfaces due to varying contact times. Static charge on the particles or the surfaces must also be considered when loading microbes in a settling chamber as charge may affect loading and distribution of the

microorganisms (Lai, 2006). To simplify loading of samples with microorganisms, we developed a system that utilizes impaction rather than settling: The Dry Aerosol Deposition Device (DADD), which is much smaller than a conventional settling chamber and allows for rapid and highly reproducible loading of samples. This report describes the design, operation, and validation of the DADD.

Dry Aerosol Deposition Device (DADD) - Description

The DADD (Figure 1) was designed to fit in a 4-foot biological safety cabinet (BSC) to contain fugitive aerosols. The aerosol is created using a single-jet Collison nebulizer (BGI Inc, Waltham, Mass.), which produces an airflow of ~ 2 liters per minute (LPM). The aerosol passes through a diffusion dryer (TSI Inc., Minneapolis, Minn.) that uses silica gel to dry the aerosol. The silica gel surrounds the circumference of the dryer, resulting in very little particle disruption. A relative humidity of 20% is achieved in the air exiting the drier. The aerosol then flows into a two-way valve (GC Valves, Charlotte, N.C.) that is controlled by an electronic switch with a sensitivity of 0.1 second. The valve defaults to the overflow position, which directs the aerosol to a high-efficiency particulate air (HEPA) filter. The collector is a single-stage (d_{50} 0.65 µm) cascade impactor (Tisch Environmental, Cleves, Ohio) that was modified to include a motor that rotates the samples at 20 rpm during loading (Figure 2). The impactor is connected to a vacuum pump that draws air at 1 cubic foot per minute (CFM). The collector is not directly attached to the two-way valve, which allows make-up air to enter the sampler at the junction. The gap is required to account for the difference in flow rates between aerosol generator (2 LPM) and the collector

(28.3 LPM). Since the device is operated in a BSC, the make-up air is HEPA- filtered and will not contain contaminants. The two-way valve and the rotating table are both activated by the same switch, which triggers the rotating to halt once sampling is complete.

Materials and Methods

Microorganisms: *Bacillus atrophaeus* (Bg) spores (ATCC 9372) were prepared to > 95% purity using standard protocols (Nicholson and Setlow, 1990). The spores were stored at -80 ºC for long-term storage and 4 ºC for short-term storage. The spores were analyzed by phase contrast microscopy to ensure they were phase bright prior to use. *Staphylococcus aureus* (ATCC 6538) was prepared by growing an overnight culture in trypticase soy broth (Becton Dickinson, Franklin Lakes, N.J.) at 37 ºC/220 rpm. The cells were harvested by centrifugation (10 min at 10,000 X *g*), then resuspended in 1% raffinose to an OD_{550} = 0.8.

Test Substrates: Glass slides were cut into 1-inch square samples, washed with ethanol, and stored in sterile containers until needed.

Aerosol exposure: Bg spores were diluted in sterile water to a concentration of $1X10^7$ CFU/mL and 35 mL of the spore solution was added to the single-jet Collison nebulizer. Compressed air (20 psi) was added to the Collison nebulizer and allowed to equilibrate for 5 minutes. Glass coupons were placed in glass Petri dishes (Tisch Environmental, Cleves, Ohio), which were loaded into the collector. Care was taken not to load coupons in the center of the dish or too close to the edge as the impactor does not contain jets in these areas. The vacuum source was turned on and the DADD was

activated to initiate sampling. The sampling was carried out for various times, after which the coupons were transferred into 50-mL centrifuge tubes containing 35 mL of neutralizing lecithin buffer (1X phosphate buffered saline, 0.5% lecithin, 0.59% sodium thiosulfate). The spores were removed from the coupons by aggressive vortexing for two minutes. The solution was serially diluted and inoculated in triplicate onto TSA plates using a WASP Spiral Plater (Microbiology International, Fredrick, Md.). The TSA plates were incubated overnight at 37 ºC, then enumerated using a Protocol automated colony counter (Microbiology International, Fredrick, Md.). The data were loaded into the *Prism-5* statistical analysis software package (GraphPad, 2236 Avenida de la Playa, La Jolla, California) and analyzed for variance.

The aerosol process for *S. aureus* was identical to that for Bg spores with the following exceptions: 1) the cells were diluted in 1% raffinose to an OD_{550} 0.8 and 2) following loading, the samples were incubated at 0-hr and 1-hr exposures to evaluate cell death due to desiccation.

Spore distribution: Glass slides were loaded with Bg spores as previously described. The slides were observed using a stereomicroscope and an upright microscope.

Results

Bg spores loaded onto glass slides with the DADD demonstrated a linear correlation between time and loading concentration (Figure 3). The 12-minute loading is a little low but the trend is clear. This indicates that loading concentration can be tuned by adjusting loading times. Reproducibility of loading is a key factor in determining the

usefulness of the technique. Repetitive loading performed at a single time point (5 min) over multiple days indicates the DADD is capable of reproducibly loading spores onto surfaces (Figure 4). The average coefficient of variation (CV) observed for spore loading within a given experiment was 13.6%. The variability is well within the acceptable range of variability found in traditional antimicrobial efficacy tests.

Loading *S. aureus* onto surfaces is more complicated because the vegetative microorganism is not as hardy as a spore and may be injured during impaction or by desiccation. To prevent desiccation, *S. aureus* was aerosolized in a solution of 1% raffinose. *S. aureus* co-aerosolized with 1% raffinose provides a high loading consistency (Figure 5). The average CV for loading triplicate samples was only 6.1%. These data demonstrate that *S. aureus* survives the impaction process. As part of this experiment, a duplicate set of glass slides was loaded with *S. aureus*, incubated at room temperature for 1 hour, and extracted. The data from this test demonstrate that, during the incubation period, an average of 13% of the cells died due to desiccation compared to control samples (Figure 6). For decontamination studies, the decrease in viability would be observed in both the test and the control population and would not bias the result. The important factor is that a significant majority of the cells remain viable during the 1-hour incubation period that is required to evaluate the performance of the decontamination technology.

Microscopic examination of Bg spores deposited onto glass slides revealed a concentric circular distribution pattern (Figure 7). The pattern is a direct result of the configuration of the impactor plate, which contains a series of jets aligned in concentric circles. The *S. aureus* cells were distributed in the same pattern as the Bg spores (data not shown).

Discussion

The DADD provides a rapid, highly reproducible means for challenging surfaces with aerosolized microorganisms. The primary reason for developing the DADD was to challenge antimicrobial/self-decontaminating/reactive materials with dry biological aerosols, as water may increase the effectiveness of these surfaces. The DADD is also well suited to contaminate carriers that are subsequently used to evaluate the efficacy of external decontamination technologies such as liquid disinfectants or UV light. The absence of water in the loading step is a key parameter in simulating threat-representative aerosols. Water rapidly evaporates from aerosolized droplets (manmade and naturally occurring) leaving droplet nuclei. The droplet nuclei are what eventually contacts the surface and will require disinfection. A settling chamber could provide the same result; however, the settling chamber requires additional time to load samples and loading variability can be difficult to control.

The use of impaction to collect microorganisms onto surfaces is not new. The cascade impactor is a common device that has been used for decades to quantify the airborne concentration of particles. However, the use of the impactor for this application is unique in that no attempt is being made to measure the airborne concentration; rather, the impactor is being used simply to load surfaces with a quantifiable amount of microorganisms. The cascade impactor typically uses collection media that are soft in nature (either agar-based media or filters) or oil-coated surfaces, which limit the amount of particle bounce, allowing for accurate sampling. For this study, a hard surface, glass, was used for the collection of microorganisms because the glass slide can easily be

observed with a light microscope. Undoubtedly, many particles simply bounce off the surface and are not collected. However, no attempt was made to measure aerosol concentration so particle bounce is of little concern for this exercise.

One potential drawback to the DADD methodology is that the spores are not uniformly distributed across the entire surface. Microscopic examination of Bg spores deposited onto glass slides shows a concentric circular distribution (Figure 7). The pattern is a direct result of the configuration of the impactor plate, which contains a series of jets aligned in concentric circles. The DADD could also be used without the rotating disc, which would deposit a series of evenly distributed spots on the coupon. In either case, the microbes are distributed in a standard pattern over the surface, but higher concentrations exist at the loading sites. Complete dispersal over the surface would be desirable and the DADD could be modified to increase distribution by varying the sample location under the jets. However, homogeneous distribution is not required to evaluate antimicrobial efficacy of the samples. The microorganisms are deposited as single particles, so their exposure to decontaminants (self contained or external) will not be affected by the loading pattern.

The DADD is a versatile device that allows for creation and deposition of aerosols onto surfaces. The characteristics of the aerosol can be changed by altering the composition of the nebulization fluid or by changing the atomizer to create larger droplets. By altering the composition of inert components in the aerosolization fluid, a specific threat can be approximated (*i.e.*, biowarfare release, respiratory transmission, etc.). For this initial study, we did not focus on trying to mimic a given threat; instead, we focused on demonstrating the reproducibility of the method.

Another important factor in the evaluation of disinfection technologies is the level of microbial agglomeration. Agglomerated microorganisms will produce a more rigorous challenge because the exterior microbes will shield the interior microbes from the decontamination agents. The DADD may provide a mechanism for loading various-sized agglomerates onto surfaces. The impactor uses plates with different-sized jets for depositing different-sized particles onto surfaces. For this study, the plate with a d_{50} of 0.65 µm was used. The d_{50} is a measure of the collection efficiency of the sampler and indicates the particle size at which the sampler has a collection efficiency of 50% (Jensen et al., 1992). The cascade impactor also has impactor plates for collecting particles ranging up to 9 µm. By using different plates, it may be possible to collect agglomerates of specific sizes. We are not aware of any other technology that can be used to load agglomerates of a specific size onto surfaces.

Summary

The DADD provides a mechanism to load coupons with a highly reproducible challenge of microorganisms. The challenge is rapid and, consequently, offers an advantage over a settling chamber. The distribution pattern of microbes onto surfaces is not completely uniform, but the DADD could be modified to increase surface distribution. The data generated in this study were based on glass coupons, but we have preliminary data indicating that the device can also be used to load such other substrates as aluminum, concrete, and fabrics. Thus the use of the DADD is limited only to what can be placed in the cascade impactor. Future studies will focus on loading microorganisms onto multiple surfaces and determining the conditions required to load specific-sized

agglomerates onto surfaces. The study of agglomerates is more difficult and the DADD may provide a unique capability for understanding how agglomerated microorganisms react with decontamination technologies.

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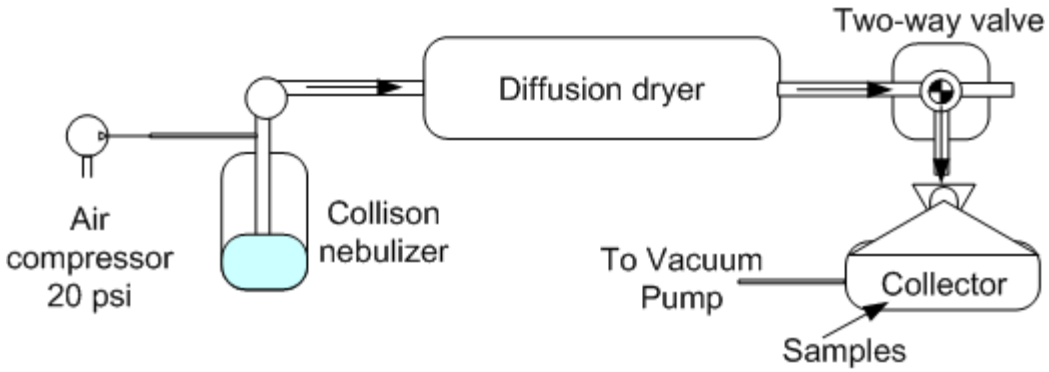


Figure 1: The Dry Aerosol Deposition Device

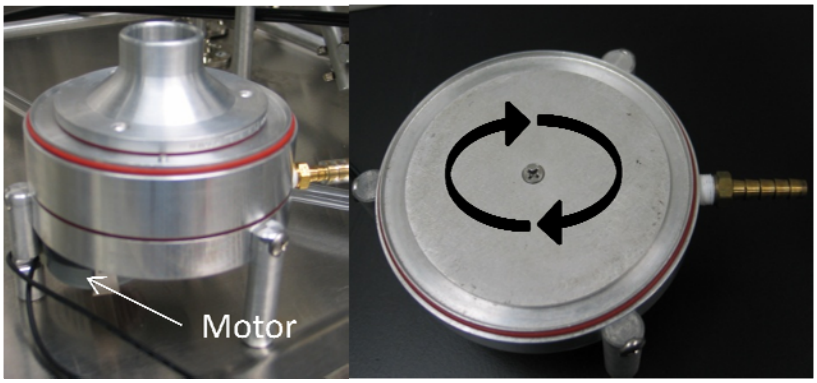
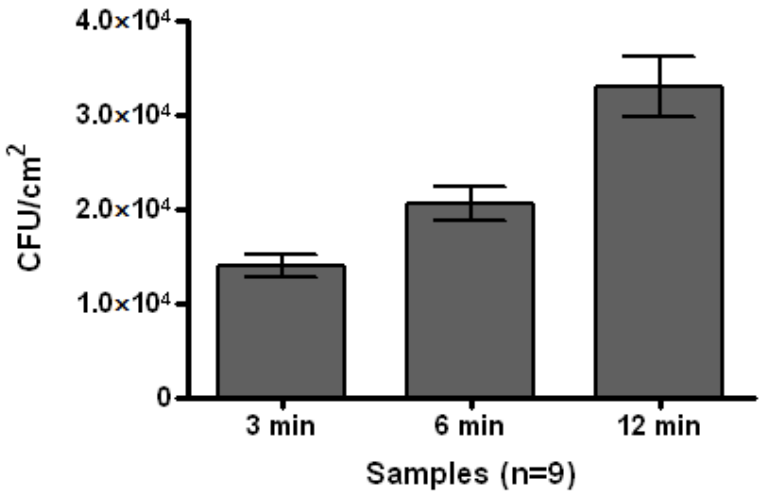


Figure 2: The DADD collector is composed of a single-stage cascade impactor that was modified to contain a spinning disc that rotates the samples during loading



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318 Figure 3: Recovery of *Bacillus atrophaeus* spores loaded onto glass coupons using the

319 Dry Aerosol Deposition Device

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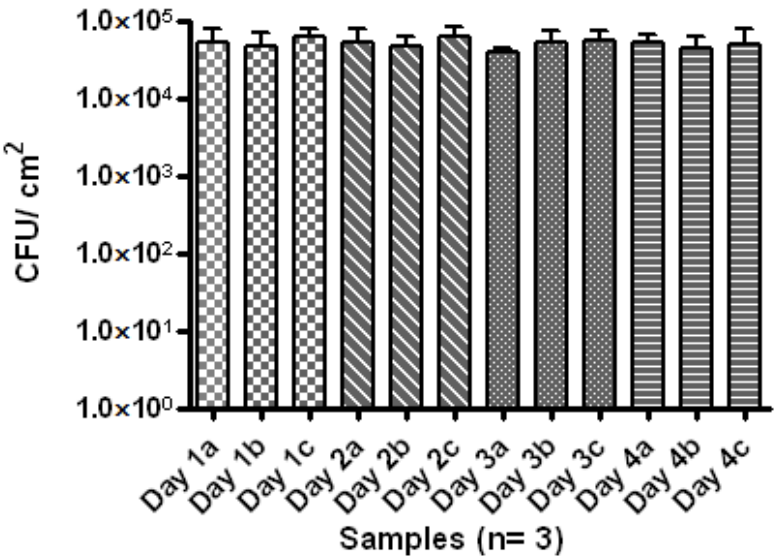

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322 Figure 4: Recovery of *Bacillus atrophaeus* spores loaded onto glass coupons using the

323 Dry Aerosol Deposition Device. Loading time was five minutes.

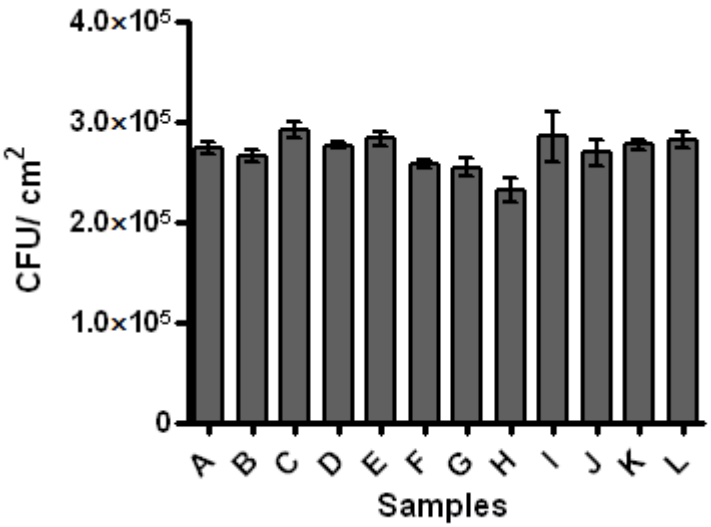

Figure 5: Recovery of *Staphylococcus aureus* loaded onto glass coupons using the Dry Aerosol Deposition Device. Loading time was five minutes.

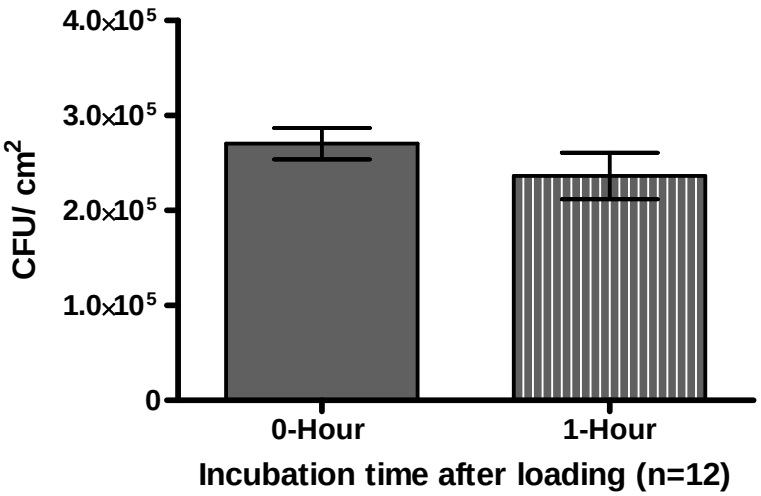

Figure 6: Recovery of *Staphylococcus aureus* loaded onto glass coupons using the Dry Aerosol Deposition Device. Loading time was five minutes.

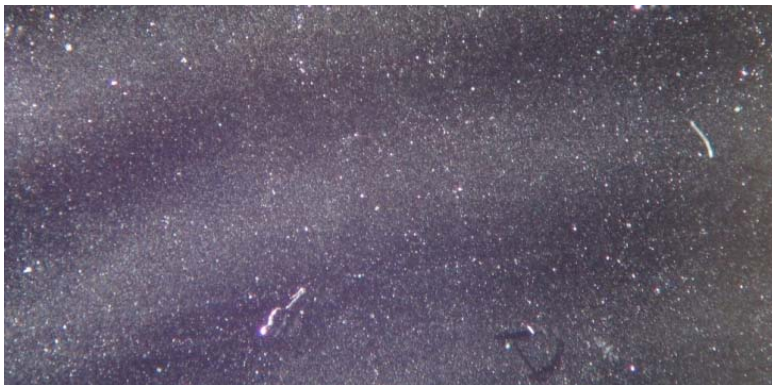

Figure 7: Distribution of *Bacillus atrophaeus s*pores loaded onto Glass Coupons using the Dry Aerosol Deposition Device.